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L5: Entry 1 of 9 File: PGPB Mar 25, 2004

DOCUMENT-IDENTIFIER: US 20040058417 A1
TITLE: Covalent joining of DNA to RNA by vaccinia topoisomerase and uses thereof

Detail Description Paragraph:
[0059] A DNA tag sequence can be attached to the isolated full-length mRNA using the methods described above. A preferred DNA tag sequence is shown in FIG. 11 both as a double stranded DNA cleavage substrate and as a covalent topoisomerase-DNA intermediate. The complementary strand of the topoisomerase-DNA intermediate includes a 3' overhang of from 1 to 4 nucleotides, which can be any mixture of adenine, guanine, cytosine or thymine, designated in the figure as N. These nucleotides will base pair with the first 1 to 4 bases of the 5' end of the isolated mRNA molecule, allowing the covalently attached topoisomerase to catalyze the transesterification reaction which joins the DNA tag to the end of the RNA sequence. The DNA tag sequence comprises a topoisomerase recognition site, preferably CCCTT, and in addition may comprise a recognition site for a site-specific restriction endonuclease, such as EcoR1, useful for the subsequent insertion of a cDNA molecule into an expression vector.

CLAIMS:

58. A method of claim 45, wherein the DNA tag sequence comprises a recognition site for a type I topoisomerase.

tags or epitope tags. Affinity purification tags are generally peptide sequences that can interact with a binding partner immobilized on a solid support. Synthetic DNA sequences encoding multiple consecutive single amino acids, such as histidine, when fused to the expressed protein, may be used for one-step purification of the recombinant protein by high affinity binding to a resin column, such as nickel sepharose. An endopeptidase recognition sequence can be engineered between the polyamino acid tag and the protein of interest to allow subsequent removal of the leader peptide by digestion with Enterokinase, and other proteases. Sequences encoding peptides such as the chitin binding domain (which binds to chitin), glutathione-S-transferase (which binds to glutathione), biotin (which binds to avidin and strepavidin), and the like can also be used for facilitating purification of the protein of interest. The affinity purification tag can be separated from the protein of interest by methods well known in the art, including the use of inteins (protein self-splicing elements, Chong, et al, Gene 192:271-281, 1997).

Detailed Description Text (64):
The strand transfer reaction pathway is diagrammed in FIG. 10A. The biotinylated DNA Substrate which contains a single topoisomerase recognition site is immobilized on the Dynabeads (Dynal) streptavidin solid support. The biotin moiety (indicated by the black square) is introduced at the 5' end of the CCCTT-containing strand via standard protocols for automated oligonucleotide synthesis. The purified vaccinia topoisomerase is reacted with the bead-bound DNA to form a covalent enzyme-DNA donor complex, as illustrated. Enzyme not bound to DNA is removed by washing the beads with buffer. The strand transfer reaction is initiated by addition of the [.sup.32 P]-CMP labeled T7 transcript which is dephosphorylated by prior treatment with alkaline phosphatase. The 5' single-strand tail of the donor complex is complementary to the 12 nucleotides at the 5' end of the T7 transcript. Religation of the covalently held biotinylated DNA strand to the T7 transcript is observed as conversion of the 30-mer RNA to a product of 50 nucleotides.

Other Reference Publication (63):
Schmitt, et al., "Affinity purification of histidine-tagged proteins," Molecular Biology Reports 81:223-230 (1993).

L5 ANSWER 1 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2004:195692 SCISEARCH
THE GENUINE ARTICLE: 774FM
TITLE: The DNA binding properties of the **Escherichia coli** RecQ helicase
AUTHOR: Dou S X; Wang P Y; Xu H Q; Xi X G (Reprint)
CORPORATE SOURCE: Ecole Normale Super, CNRS, UMR 8113, Lab Biotechnol & Pharmacol Genet Appl, 61 Ave President Wilson, F-94235 Cachan, France (Reprint); Ecole Normale Super, CNRS, UMR 8113, Lab Biotechnol & Pharmacol Genet Appl, F-94235 Cachan, France; Chinese Acad Sci, Inst Phys, Lab Soft Matter Phys, Beijing 100080, Peoples R China
COUNTRY OF AUTHOR: France; Peoples R China
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (20 FEB 2004) Vol. 279, No. 8, pp. 6354-6363.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 52

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The RecQ helicase family is highly conserved from bacteria to men and plays a conserved role in the preservation of genome integrity. Its deficiency in human cells leads to a marked genomic instability that is associated with premature aging and cancer. To determine the thermodynamic parameters for the interaction of **Escherichia coli** RecQ helicase with DNA, equilibrium binding studies have been performed using the thermodynamic rigorous fluorescence titration technique. Steady-state fluorescence anisotropy measurements of fluorescein-labeled oligonucleotides revealed that RecQ helicase bound to DNA with an apparent binding stoichiometry of 1 protein monomer/10 nucleotides. This stoichiometry was not altered in the presence of AMPPNP (adenosine 5'-(beta,gamma-imido) triphosphate) or ADP. Analyses of RecQ helicase interactions with oligonucleotides of different lengths over a wide range of pH, NaCl, and nucleic acid concentrations indicate that the RecQ helicase has a single strong DNA binding site with an association constant at 25 degreesC of K = 6.7 +/- 0.95 x 10(6) M-1 and a cooperativity parameter of omega = 25.5 +/- 1.2. Both **single-stranded DNA** and double-stranded DNA bind competitively to the same site. The intrinsic affinities are salt-dependent, and the formation of DNA-helicase complex is accompanied by a net release of 3-4 ions. Allosteric effects of nucleotide cofactors on RecQ binding to DNA were observed only for **single-stranded DNA** in the presence of 1.5 mm AMPPNP, whereas both AMPPNP and ADP had no detectable effect on double-stranded DNA binding over a large range of nucleotide cofactor concentrations.

L5 ANSWER 2 OF 16 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003488948 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12909639
TITLE: RecQ helicase stimulates both DNA catenation and changes in DNA topology by **topoisomerase III**.
AUTHOR: Harmon Frank G; Brockman Joel P; Kowalczykowski Stephen C
CORPORATE SOURCE: Division of Biological Sciences, Section of Microbiology, Center for Genetics and Development, University of California-Davis, 1 Shields Avenue, Davis, CA 95616, USA.
SOURCE: Journal of biological chemistry, (2003 Oct 24) 278 (43) 42668-78.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200401
ENTRY DATE: Entered STN: 20031021
Last Updated on STN: 20040106
Entered Medline: 20040105

AB Together, RecQ helicase and **topoisomerase III** (Topo III) of **Escherichia coli** comprise a potent DNA strand passage activity that can catenate covalently closed DNA (Harmon, F. G., DiGate, R. J., and Kowalczykowski, S. C. (1999) Mol. Cell 3, 611-620). Here we directly assessed the structure of the catenated DNA species formed by RecQ helicase and Topo III using atomic force microscopy. The images show complex catenated DNA species involving crossovers between multiple double-stranded DNA molecules that are consistent with full catenanes. **E. coli single-stranded DNA**-binding protein significantly stimulated both the topoisomerase activity of Topo III alone and the DNA strand passage activity of RecQ helicase and Topo III. Titration data suggest that an intermediate of the RecQ helicase unwinding process, perhaps a RecQ helicase-DNA fork, is the target for Topo III action. Catenated DNA is the predominant product under conditions of molecular crowding; however, we also discovered that RecQ helicase and **single-stranded DNA**-binding protein greatly stimulated the intramolecular strand passage ("supercoiling") activity of Topo III, as revealed by changes in the linking number of uncatenated DNA. Together our results demonstrate that RecQ helicase and Topo III function together to comprise a potent and concerted **single-strand DNA** passage activity that can mediate both catenation-decatenation processes and changes in DNA topology.

L5 ANSWER 3 OF 16 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001408241 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11459965
TITLE: A yeast gene, MGS1, encoding a DNA-dependent AAA(+) ATPase is required to maintain genome stability.
AUTHOR: Hishida T; Iwasaki H; Ohno T; Morishita T; Shinagawa H
CORPORATE SOURCE: Department of Molecular Microbiology, Research Institute for Microbial Diseases, Osaka University, Yamadaoka 3-1, Suita, Osaka 565-0871, Japan.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2001 Jul 17) 98 (15) 8283-9. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010903
Last Updated on STN: 20010903
Entered Medline: 20010830

AB Changes in DNA superhelicity during DNA replication are mediated primarily by the activities of DNA helicases and topoisomerases. If these activities are defective, the progression of the replication fork can be hindered or blocked, which can lead to double-strand breaks, elevated recombination in regions of repeated DNA, and genome instability. Hereditary diseases like Werner's and Bloom's Syndromes are caused by defects in DNA helicases, and these diseases are associated with genome instability and carcinogenesis in humans. Here we report a Saccharomyces cerevisiae gene, MGS1 (Maintenance of Genome Stability 1), which encodes a protein belonging to the AAA(+) class of ATPases, and whose central region is similar to **Escherichia coli** RuvB, a Holliday junction branch migration motor protein. The Mgs1 orthologues are highly conserved in prokaryotes and eukaryotes. The Mgs1 protein possesses DNA-dependent ATPase and **single-strand DNA** annealing activities. An mgs1 deletion mutant has an elevated rate of

mitotic recombination, which causes genome instability. The mgs1 mutation is synergistic with a mutation in **top3** (encoding **topoisomerase III**), and the double mutant exhibits severe growth defects and markedly increased genome instability. In contrast to the mgs1 mutation, a mutation in the sgs1 gene encoding a DNA helicase homologous to the Werner and Bloom helicases suppresses both the growth defect and the increased genome instability of the **top3** mutant. Therefore, evolutionarily conserved Mgs1 may play a role together with RecQ family helicases and DNA topoisomerases in maintaining proper DNA topology, which is essential for genome stability.

L5 ANSWER 4 OF 16 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001372899 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11429611
TITLE: Crystal structure of a complex of a type IA DNA topoisomerase with a **single-stranded DNA** molecule.
AUTHOR: Changela A; DiGate R J; Mondragon A
CORPORATE SOURCE: Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60208, USA.
SOURCE: Nature, (2001 Jun 28) 411 (6841) 1077-81.
Journal code: 0410462. ISSN: 0028-0836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-117D
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010723
Last Updated on STN: 20010723
Entered Medline: 20010719

AB A variety of cellular processes, including DNA replication, transcription, and chromosome condensation, require enzymes that can regulate the ensuing topological changes occurring in DNA. Such enzymes-DNA topoisomerases-alter DNA topology by catalysing the cleavage of **single-stranded DNA** (ssDNA) or double-stranded DNA (dsDNA), the passage of DNA through the resulting break, and the rejoining of the broken phosphodiester backbone. DNA **topoisomerase III** from **Escherichia coli** belongs to the type IA family of DNA topoisomerases, which transiently cleave ssDNA via formation of a covalent 5' phosphotyrosine intermediate. Here we report the crystal structure, at 2.05 A resolution, of an inactive mutant of **E. coli** DNA **topoisomerase III** in a non-covalent complex with an 8-base ssDNA molecule. The enzyme undergoes a conformational change that allows the oligonucleotide to bind within a groove leading to the active site. We note that the ssDNA molecule adopts a conformation like that of B-DNA while bound to the enzyme. The position of the DNA within the realigned active site provides insight into the role of several highly conserved residues during catalysis. These findings confirm various aspects of the type IA topoisomerase mechanism while suggesting functional implications for other topoisomerases and proteins that perform DNA rearrangements.

L5 ANSWER 5 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2000:688554 SCISEARCH
THE GENUINE ARTICLE: 351EL
TITLE: Interaction between yeast Sgs1 helicase and DNA **topoisomerase III**
AUTHOR: Bennett R J; NoirotGros M F; Wang J C (Reprint)
CORPORATE SOURCE: HARVARD UNIV, DEPT MOL & CELLULAR BIOL, 7 DIVIN AVE, CAMBRIDGE, MA 02138 (Reprint); HARVARD UNIV, DEPT MOL & CELLULAR BIOL, CAMBRIDGE, MA 02138

COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1 SEP 2000) Vol. 275, No. 35, pp. 26898-26905.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 39
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The Saccharomyces cerevisiae Sgs1 protein is a member of the RecQ family of DNA helicases that includes the human Bloom's syndrome and Werner's syndrome proteins. In this work, we report studies on the interaction between Sgs1 and DNA **topoisomerase III** in vitro and in vivo, Affinity chromatography experiments with various fragments of Sgs1, a 1447-amino acid polypeptide, suggested that its N-terminal one-fifth was sufficient for interaction with DNA **topoisomerase III**. Gel electrophoretic mobility shift assays also indicated that a fragment Sgs1(1-283), containing residues 1-283, inhibited the binding of DNA **topoisomerase III** to **single-stranded DNA**. A shorter protein fragment containing residues 1-107 also showed partial inhibition in these assays. Studies of a sgs1 top1 double mutant lacking both Sgs1 and DNA topoisomerase I showed that the slow growth phenotype of this double mutant is suppressed by expressing full-length Sgs1, but not Sgs1 without the N-terminal 107 amino acid residues. In sgs1 **top3** cells devoid of DNA **topoisomerase III**, however, expression of full-length Sgs1 or Sgs1 lacking the N-terminal 107 amino acid residues has the same effect of reducing the growth rate of the double mutant. These in vitro and in vivo data indicate that Sgs1 and DNA **topoisomerase III** physically interact and that this interaction is physiologically significant.

L5 ANSWER 6 OF 16 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1999205900 EMBASE
TITLE: Purification and characterization of human DNA **topoisomerase IIIα**.
AUTHOR: Goulaouic H.; Roulon T.; Flamand O.; Grondard L.; Lavelle F.; Riou J.-F.
CORPORATE SOURCE: H. Goulaouic, Rhone-Poulenc Rorer, Centre recherche Vitry-Alfortville, 13 quai Jules Guesde, 94403 Vitry sur Seine Cedex, France. helene.goulaouic@rp-rorer.fr
SOURCE: Nucleic Acids Research, (15 Jun 1999) 27/12 (2443-2450).
Refs: 31
ISSN: 0305-1048 CODEN: NARHAD
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Human **topoisomerase IIIα** (hTopo IIIα), the recently identified first member of the topoisomerase IA subfamily in humans, has a central domain which is highly homologous to the yeast **topoisomerase III**, but an overall organization closer to that of **Escherichia coli** DNA topoisomerase I. In order to determine the properties of hTopo IIIα, compared to those of other topoisomerase IA subfamily members, we purified this enzyme to near homogeneity, together with an active site-mutant Y337F. We show that hTopo IIIα is able to relax negatively supercoiled DNA in a distributive manner, leading to the total disappearance of the initial substrate and the appearance of intermediate topoisomers. This DNA relaxation activity

is magnesium-dependent, although a low concentration of MgCl2 is sufficient to obtain efficient catalysis. 32P-transfer experiments demonstrated that hTopo IIIα is able to cleave a single-stranded oligonucleotide and to bind covalently to the 5'-end of the cleaved DNA. Addition of 0.5 M NaCl reverses the reaction, leading to the religation of the oligonucleotide. Experiments utilizing several different single-stranded oligonucleotides permitted us to map several cleavage sites and to deduce a consensus sequence for DNA cleavage (CANNN↓), which is different from that for other members of the Topo IA subfamily.

L5 ANSWER 7 OF 16 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2000045187 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10574789
TITLE: The structure of **Escherichia coli** DNA **topoisomerase III**.
AUTHOR: Mondragon A; DiGate R
CORPORATE SOURCE: Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208-3500, USA.. a-mondragon@nwu.edu
CONTRACT NUMBER: GM48445 (NIGMS)
GM51350 (NIGMS)
SOURCE: Structure with Folding & design, (1999 Nov 15) 7 (11) 1373-83.
Journal code: 100889329. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1D6M
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20021030
Entered Medline: 19991214

AB BACKGROUND: DNA topoisomerases are enzymes that change the topology of DNA. Type IA topoisomerases transiently cleave one DNA strand in order to pass another strand or strands through the break. In this manner, they can relax negatively supercoiled DNA and catenate and decatenate DNA molecules. Structural information on **Escherichia coli** DNA **topoisomerase III** is important for understanding the mechanism of this type of enzyme and for studying the mechanistic differences among different members of the same subfamily. RESULTS: The structure of the intact and fully active **E. coli** DNA **topoisomerase III** has been solved to 3.0 A resolution. The structure shows the characteristic fold of the type IA topoisomerases that is formed by four domains, creating a toroidal protein. There is remarkable structural similarity to the 67 kDa N-terminal fragment of **E. coli** DNA topoisomerase I, although the relative arrangement of the four domains is significantly different. A major difference is the presence of a 17 amino acid insertion in **topoisomerase III** that protrudes from the side of the central hole and could be involved in the catenation and decatenation reactions. The active site is formed by highly conserved amino acids, but the structural information and existing biochemical and mutagenesis data are still insufficient to assign specific roles to most of them. The presence of a groove in one side of the protein is suggestive of a **single-stranded DNA** (ssDNA)-binding region. CONCLUSIONS: The structure of **E. coli** DNA **topoisomerase III** resembles the structure of **E. coli** DNA topoisomerase I except for the presence of a positively charged loop that may be involved in catenation and decatenation. A groove on the side of the protein leads to the active site and is likely to be involved in DNA binding. The structure helps to establish the overall mechanism for the type IA subfamily of topoisomerases with greater confidence and expands the structural basis

for understanding these proteins.

L5 ANSWER 8 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:256931 CAPLUS
DOCUMENT NUMBER: 131:112967
TITLE: Overexpression and purification of **Escherichia coli** DNA **topoisomerase III**
AUTHOR(S): DiGate, Russell J.
CORPORATE SOURCE: Department of Biomedical Chemistry, School of Pharmacy, University of Maryland at Baltimore, Baltimore, MD, USA
SOURCE: Methods in Molecular Biology (Totowa, New Jersey) (1999), 94(DNA Topoisomerase Protocols, Vol. 1), 153-162
CODEN: MMBIED; ISSN: 1064-3745
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A protocol for the purification of DNA **topoisomerase III** (topo III) of **Escherichia coli** is outlined. The procedure uses soybean trypsin inhibitor agarose, DE-52 cellulose batch, and **single-stranded DNA** cellulose chromatogs. and allows for purification of relatively large quantities of topo III in 3-4 day time period.
REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1998:311662 SCISEARCH
THE GENUINE ARTICLE: ZH610
TITLE: Purification and characterization of the Sgs1 DNA helicase activity of Saccharomyces cerevisiae
AUTHOR: Bennett R J; Sharp J A; Wang J C (Reprint)
CORPORATE SOURCE: HARVARD UNIV, DEPT MOL & CELLULAR BIOL, 7 DIVIN AVE, CAMBRIDGE, MA 02138 (Reprint); HARVARD UNIV, DEPT MOL & CELLULAR BIOL, CAMBRIDGE, MA 02138
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (17 APR 1998) Vol. 273, No. 16, pp. 9644-9650.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 38
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The yeast Saccharomyces cerevisiae Sgs1 protein is a member of a family of DNA helicases that include the **Escherichia coli** RecQ protein and the products of human Bloom's syndrome and Werner's syndrome genes. To study the enzymatic characteristics of the protein, a recombinant Sgs1 fragment (amino acids 400-1268 of the 1447-amino acid full-length protein) was overexpressed in yeast and purified to near homogeneity. The purified protein exhibits an ATPase activity in the presence of single- or double-stranded DNA. In the presence of ATP or dATP, unwinding of duplex DNA or a DNA-RNA heteroduplex by the recombinant Sgs1 fragment was readily observed. Similar to the **E. coli** RecQ helicase, displacement of the DNA strand occurs in the 3' to 5' direction with respect to the **single-stranded DNA** flanking the duplex. The efficiency of unwinding was found to correlate inversely with the length of the duplex region and was enhanced by the presence off. coli **single-stranded DNA** binding protein. In addition, the recombinant Sgs1 fragment was found to bind more tightly to a forked DNA substrate than to either single-or

double-stranded DNA.

L5 ANSWER 10 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 96:661945 SCISEARCH
THE GENUINE ARTICLE: VF614
TITLE: AN RNA TOPOISOMERASE
AUTHOR: WANG H; DIGATE R J; SEEMAN N C (Reprint)
CORPORATE SOURCE: NYU, DEPT CHEM, NEW YORK, NY, 10003 (Reprint); NYU, DEPT CHEM, NEW YORK, NY, 10003; UNIV MARYLAND, MARYLAND BIOTECHNOL INST, CTR MED BIOTECHNOL, BALTIMORE, MD, 21201; UNIV MARYLAND, MARYLAND BIOTECHNOL INST, DEPT PHARMACEUT SCI, SCH PHARM, BALTIMORE, MD, 21201
COUNTRY OF AUTHOR: USA
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (03 SEP 1996) Vol. 93, No. 18, pp. 9477-9482.
ISSN: 0027-8424.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 33
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A synthetic strand of RNA has been designed so that it can adopt two different topological states (a circle and a trefoil knot) when ligated into a cyclic molecule. The RNA knot and circle have been characterized by their behavior in gel electrophoresis and sedimentation experiments, This system allows one to assay for the existence of an RNA topoisomerase, because the two RNA molecules can be interconverted only by a strand passage event. We find that the interconversion of these two species can be catalyzed by **Escherichia coli** DNA **topoisomerase III**, indicating that this enzyme can act as an RNA topoisomerase. The conversion of circles to knots is accompanied by a small amount of RNA catenane generation. These findings suggest that strand passage must be considered a potential component of the folding and modification of RNA structures.

L5 ANSWER 11 OF 16 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 96224127 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8621552
TITLE: The role of the carboxyl-terminal amino acid residues in **Escherichia coli** DNA **topoisomerase III**-mediated catalysis.
AUTHOR: Zhang H L; Malpure S; Li Z; Hiasa H; DiGate R J
CORPORATE SOURCE: Molecular and Cell Biology Program, University of Maryland, Baltimore, Maryland 21201, USA.
CONTRACT NUMBER: GM48445 (NIGMS)
SOURCE: Journal of biological chemistry, (1996 Apr 12) 271 (15) 9039-45.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960627
Last Updated on STN: 19960627
Entered Medline: 19960620

AB The role that the carboxyl-terminal amino acids of **Escherichia coli** DNA topoisomerase I (Topo I) and III (Topo III) play in catalysis was examined by comparing the properties of Topo III with those of a truncated enzyme lacking the generalized DNA binding domain of Topo III, Topo I, and a hybrid topoisomerase polypeptide containing the amino-terminal 605 amino acids of Topo III and the putative generalized DNA binding domain of Topo I. The deletion of the carboxyl-terminal 49

amino acids of Topo III decreases the affinity of the enzyme for its substrate, **single-stranded DNA**, by approximately 2 orders of magnitude and reduces Topo III-catalyzed relaxation of supercoiled DNA and Topo III-catalyzed resolution of DNA replication intermediates to a similar extent. Fusion of the carboxyl-terminal 312 amino acid residues of Topo I onto the truncated molecule stimulates topoisomerase-catalyzed relaxation 15-20-fold, to a level comparable with that of full-length Topo III. However, topoisomerase-catalyzed resolution of DNA replication intermediates was only stimulated 2-3-fold. Therefore, the carboxyl-terminal amino acids of these topoisomerases constitute a distinct and separable domain, and this domain is intimately involved in determining the catalytic properties of these polypeptides.

L5 ANSWER 12 OF 16 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 96007520 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7559540
TITLE: **Escherichia coli** DNA **topoisomerase III** is a site-specific DNA binding protein that binds asymmetrically to its cleavage site.
AUTHOR: Zhang H L; Malpure S; DiGate R J
CORPORATE SOURCE: Molecular and Cell Biology Program, University of Maryland at Baltimore 21201, USA.
CONTRACT NUMBER: GM48445-02 (NIGMS)
SOURCE: Journal of biological chemistry, (1995 Oct 6) 270 (40) 23700-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199511
ENTRY DATE: Entered STN: 19951227
Last Updated on STN: 19951227
Entered Medline: 19951114

AB The binding of DNA **topoisomerase III** (Topo III) to a **single-stranded DNA** substrate containing a strong cleavage site has been examined. The minimal substrate requirement for Topo III-catalyzed cleavage has been determined to consist of 7 bases; 6 bases 5' to the cleavage site and only 1 base 3' to the site. Nuclease P1 protection experiments indicate that the enzyme also binds to its substrate asymmetrically, protecting approximately 12 bases 5' to the cleavage site and only 2 bases 3' to the cleavage site. A catalytically inactive mutant of Topo III shows the same protection pattern as the active polypeptide, indicating that Topo III is a site-specific binding protein as well as a topoisomerase. Consistent with this view, an oligonucleotide containing a cleavage site is a more effective inhibitor and is bound more efficiently by Topo III than an oligonucleotide without a cleavage site.

L5 ANSWER 13 OF 16 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 94179321 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7510701
TITLE: The carboxyl-terminal residues of **Escherichia coli** DNA **topoisomerase III** are involved in substrate binding.
COMMENT: Erratum in: J Biol Chemical 1995 Sep 1;270(35):20870. PubMed ID: 7657674
AUTHOR: Zhang H L; DiGate R J
CORPORATE SOURCE: Molecular and Cell Biology Program, University of Maryland at Baltimore 21201.
CONTRACT NUMBER: GM48445 (NIGMS)
RR05770-14 (NCRR)

SOURCE: Journal of biological chemistry, (1994 Mar 25) 269 (12) 9052-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940428
Last Updated on STN: 19960315
Entered Medline: 19940421

AB The nucleic acid-binding domain of **Escherichia coli** DNA **topoisomerase III** (Topo III) has been identified using a selection procedure designed to isolate inactive Topo III polypeptides. Deletion of this binding domain, contained in the carboxyl terminus of Topo III, results in a drastic reduction in the ability of the enzyme to bind to **single-stranded DNA** and RNA substrates. Successive truncation of the enzyme within this region results in the gradual loss of nucleic acid binding activity and in a gradual change in the mechanism of Topo III-catalyzed relaxation of negatively supercoiled DNA. The reduction of nucleic acid binding activity of the truncated polypeptides does not result in a loss of cleavage site specificity for the enzyme, suggesting that other amino acids are involved in the positioning of the nucleic acid within the nicking/closing site of the topoisomerase.

L5 ANSWER 14 OF 16 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 92381032 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1324925
TITLE: Identification of the yeast **TOP3** gene product as a single strand-specific DNA topoisomerase.
AUTHOR: Kim R A; Wang J C
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138-2092.
CONTRACT NUMBER: CA47958 (NCI)
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SOURCE: Journal of biological chemistry, (1992 Aug 25) 267 (24) 17178-85.
Journal code: 2985121R. ISSN: 0021-9258.
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AB The **TOP3** gene of the yeast Saccharomyces cerevisiae was postulated to encode a DNA topoisomerase, based on its sequence homology to **Escherichia coli** DNA topoisomerase I and the suppression of the poor growth phenotype of **top3** mutants by the expression of the **E. coli** enzyme (Wallis, J.W., Chrebet, G., Brodsky, G., Golfe, M., and Rothstein, R. (1989) Cell 58, 409-419). We have purified the yeast **TOP3** gene product to near homogeneity as a 74-kDA protein from yeast cells lacking DNA topoisomerase I and overexpressing a plasmid-borne **TOP3** gene linked to a phosphate-regulated yeast PHO5 gene promoter. The purified protein possesses a distinct DNA topoisomerase activity: similar to **E. coli** DNA topoisomerases I and III, it partially relaxes negatively but not positively supercoiled DNA. Several experiments, including the use of a negatively supercoiled heteroduplex DNA containing a 29-nucleotide single-stranded loop, indicate that the activity has a strong preference for **single-stranded DNA**.
A protein-DNA covalent complex in which the 74-kDa protein is linked to a

5' DNA phosphoryl group has been identified, and the nucleotide sequences of 30 sites of DNA-protein covalent complex formation have been determined. These sequences differ from those recognized by **E. coli** DNA topoisomerase I but resemble those recognized by **E. coli** DNA **topoisomerase III**.
Based on these results, the yeast **TOP3** gene product can formally be termed S. cerevisiae DNA **topoisomerase III**.
Analysis of supercoiling of intracellular yeast plasmids in various DNA topoisomerase mutants indicates that yeast DNA **topoisomerase III** has at most a weak activity in relaxing negatively supercoiled double-stranded DNA in vivo, in accordance with the characteristics of the purified enzyme.

L5 ANSWER 15 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 91:380018 SCISEARCH
THE GENUINE ARTICLE: FV180
TITLE: DNA **TOPOISOMERASE-III** FROM EXTREMELY THERMOPHILIC ARCHAEBACTERIA - ATP-INDEPENDENT TYPE-I TOPOISOMERASE FROM DESULFUROCOCCUS-AMYLOLYTICUS DRIVES EXTENSIVE UNWINDING OF CLOSED CIRCULAR DNA AT HIGH-TEMPERATURE
AUTHOR: SLESAREV A I (Reprint); ZAITZEV D A; KOPYLOV V M; STETTER K O; KOZYAVKIN S A
CORPORATE SOURCE: UNIV REGENSBURG, LEHRSTUHL MIKROBIOL, UNIV STR 31, W-8400 REGENSBURG, GERMANY (Reprint); ACAD SCI USSR, INST MOLEC GENET, MOSCOW 123182, USSR; RE KAVETSKY ONCOL PROBLEMS INST, KIEV 252127, UKRAINE, USSR
COUNTRY OF AUTHOR: GERMANY; USSR
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1991) Vol. 266, No. 19, pp. 12321-12328.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 73
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A second type I topoisomerase was purified from the extremely thermophilic archaebacterium Desulfurococcus amylolyticus. In contrast to the previously described reverse gyrase from this organism, the novel enzyme designated as Dam **topoisomerase III** is an ATP-independent relaxing topoisomerase. It is a monomer with M(r) 108,000, as determined by electrophoresis under denaturing conditions and by size exclusion chromatography. Dam **topoisomerase III**, like other bacterial type I topoisomerases, absolutely requires Mg2+ for activity and is specific for **single-stranded DNA**. At 60-80-degrees-C, it relaxes negatively but not positively supercoiled DNA and is inhibited by single-stranded M13 DNA. At 95-degrees-C, the enzyme unwinds both positively and negatively supercoiled substrates and produces extensively unwound form I* and I** DNA. The peculiarities of DNA topoisomerization at high temperatures are discussed.

L5 ANSWER 16 OF 16 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 84203564 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6326814
TITLE: **Escherichia coli** DNA **topoisomerase III**: purification and characterization of a new type I enzyme.
AUTHOR: Srivenugopal K S; Lockshon D; Morris D R
SOURCE: Biochemistry, (1984 Apr 24) 23 (9) 1899-906. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

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AB A new topoisomerase capable of relaxing negatively supercoiled DNA in **Escherichia coli** has been identified during chromatography on novobiocin-Sepharose. A simple and reproducible purification procedure is described to obtain this enzyme, called **topoisomerase III** (topo III), in a homogeneous form. The protein is a single polypeptide with a molecular weight of 74 000 +/- 2000 and is a type I topoisomerase, changing the linking number of DNA circles in steps of one. It is present in deletion strains lacking the topA gene and further differs from the well-studied topoisomerase I (omega protein; Eco topo I) in (1) its requirement for K+ in addition to Mg2+ to exhibit optimal activity and (2) its affinity to novobiocin-Sepharose. Positively supercoiled DNA is not relaxed during exposure to the enzyme. Topo III has no ATPase activity, and ATP does not show any discernible effect on the reduction of superhelical turns. The purified topoisomerase has no supercoiling activity and is unaffected by high concentrations of oxolinic acid and novobiocin in the relaxing reaction. **Single-stranded DNA** and spermidine strongly inhibit the topoisomerase activity.

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